

proliferation and maintenance of adjacent follicular stem cells and the differentiation of their progeny to specify the size, morphology and pigmentation of the hair shaft. Furthermore, signaling to the DP from surrounding keratinocytes coordinates the activities of follicular stem cells and the dermal papilla to orchestrate morphogenesis. These studies complement analysis of genetic manipulations in follicular stem cells to provide detailed insight into the interactions between epithelial stem cells and their mesenchymal niche in an adult mammal.

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#### Program/Abstract # 299

##### **Pax6 is required for neuronal progenitor cell proliferation during cone cell regeneration**

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Pax6 is required for proper development of the vertebrate eye. Unlike mammals, the adult zebrafish retina possesses a robust regenerative response. We tested whether Pax6 was required for rod and cone photoreceptor regeneration in the light-damaged adult zebrafish retina. In this model, rod precursor and Müller glial cell divisions precede regeneration of the photoreceptors. Pax6-positive neuronal progenitors arise from the Müller glia, continue to divide, and migrate along the Müller glial processes to the outer nuclear layer where they differentiate into the lost photoreceptors. Zebrafish contain two *pax6* genes, *pax6a* and *pax6b*, that are functionally redundant in retinal development. To study the role of Pax6 proteins during retinal regeneration, we developed a method to conditionally inhibit protein expression by *in vivo* electroporation of a cocktail of anti-*pax6a* and anti-*pax6b* morpholinos into the regenerating retina. The *pax6a/6b* morphant retina exhibited reduced Pax6a and Pax6b protein expression. While the *pax6a/6b* morphant did not exhibit inhibition of rod precursor or Müller glial cell division, it did exhibit inhibition of Müller glial-derived neuronal progenitor proliferation. This subsequently inhibited cone photoreceptor regeneration. However, increased levels of rod precursor cell proliferation lead to a complete regeneration of rod photoreceptors, which is independent of Müller glial-derived neuronal progenitors. Thus, Pax6 is required for neuronal progenitor cell amplification during cone cell regeneration in the light-damaged adult retina.

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#### Program/Abstract # 300

##### **Live cell imaging of the zebrafish dermomyotome**

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The zebrafish dermomyotome contains a population of myogenic progenitors that contributes to growth of the myotome and possibly to the adult muscle stem cell population. We have characterized a unique line of transgenic zebrafish expressing GFP in the dermomyotome. Shortly before 24 h we find distinct expression of GFP in a pattern strongly reminiscent of the expression of Pax7 in myogenic progenitors. We have confirmed the identity of these cells as myogenic progenitors by colocalization of GFP and Pax7. We

have also found changes in expression of GFP that mimic changes in Pax7 expression when altering Hedgehog signaling. We are now using this transgenic line as a tool to study novel aspects of dermomyotome development. The longevity of GFP has given us insight into the descendants of these myogenic progenitors. We have found GFP positive, small diameter muscle fibers, which express slow myosin, suggesting that the dermomyotome may give rise to slow fibers as well as fast fibers. We have also found that the sparse cells of the dermomyotome dispersed on the lateral surface of the myotome extend projections and make connections with one another. This morphology suggests possibilities for direct communication between cells within the dermomyotome. Finally this line allows us to follow myogenic cell behaviors in real time using time lapse microscopy. So far we have been able to confirm that this population is actively dividing. We believe this transgenic line provides a distinctive tool in studies of myogenesis.

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#### Program/Abstract # 301

##### **Culture of primary myogenic cells derived from adult muscle and electric organ of the gymnotiform *S. macrurus***

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We have established primary myogenic precursor cell cultures from adult skeletal muscle and the muscle derived electric organ (EO) of the gymnotiform *S. macrurus*. Our protocol was modified from those of Fauconneau et. al., (2000) for the isolation of rainbow trout myoblasts. Briefly, skeletal muscle and EO were dissected from adult fish, cut into 5 mm<sup>3</sup> chunks and incubated overnight in growth medium (GM: 10% FBS/1% penicillin and streptomycin in L-15 medium). Tissue was then minced to 1 mm<sup>3</sup> pieces and treated with collagenase and trypsin for enzymatic dissociation. Following centrifugation, cells were plated on collagen-coated wells and maintained to confluence in GM. Myogenic cell differentiation was induced by switching GM to differentiation medium (DM: 2% FBS/1% penicillin and streptomycin in L-15 medium) for 5 days. We detected both mono and multinucleated cells that were immunolabeled with antibodies against mature muscle markers desmin, titin, and sarcomeric MHC. This differentiation capacity was observed even after several passages. These data represent the first known isolation of myogenic precursor cells from any electric fish muscle, as well as their differentiation *in vitro*. Establishment of a *S. macrurus* myogenic precursor cell line will permit investigations of molecular and cellular mechanisms involved in the regeneration of muscle and its derived tissues. Further, a myogenic precursor cell line will facilitate our studies on the developmental origin of EO.

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#### Program/Abstract # 302

##### **Identifying the precursor zone of muscle satellite cells in *Xenopus laevis* embryos**

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